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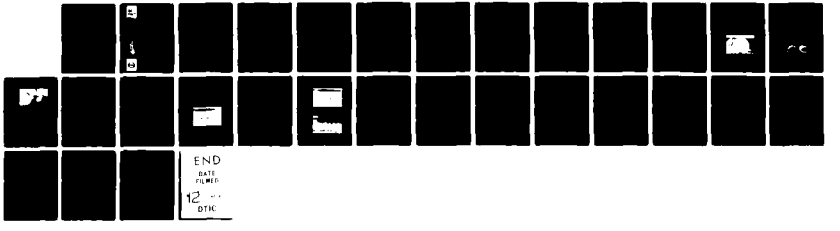

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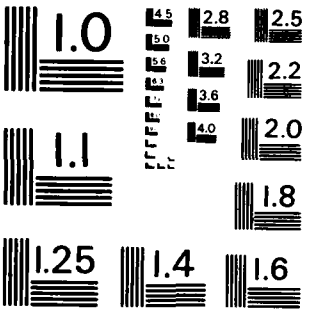

US Army Corps of Engineers



**AQUATIC PLANT CONTROL RESEARCH PROGRAM**

MISCELLANEOUS PAPER A-83-4

# MICROBIOLOGICAL CONTROL OF EURASIAN WATERMILFOIL

by

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Final Report

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Cellulolytic and pectinolytic microorganisms were isolated from the microbial populations naturally resident in the phyllosphere of Eurasian watermilfoil, *Myriophyllum spicatum* and of *M. heterophyllum*. The yield of their respective operative enzymes was maximized by growth in appropriate cellulose and pectin media; the organisms, when subsequently applied to the plants, accelerated the plants' necrosis and decline. That cellulose and

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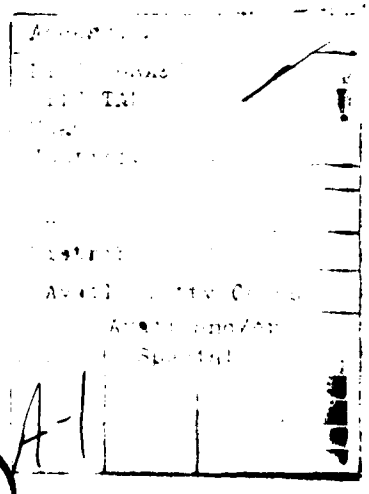
pectin are particularly vulnerable target tissues in *Myriophyllum* spp. was confirmed by the significant increase in plant necrosis achieved over untreated controls by the simple addition of sterile cellulose and pectin media to respective test chambers. Presumably this reflected the selective stimulus provided by these substrates to the resident cellulolytic and pectinolytic microflora.

A consortium of cyanobacteria associated with *Myriophyllum* was also found to accelerate necrosis, as did its sterile growth medium; again, presumably, as a reflection of the selective stimulus provided to the cyanobacteria in the phyllosphere.

The species determination of phyllosphere residents was reflected in the significantly higher pathogenic potential of the isolates from *M. spicatum* to that species than to *M. heterophyllum*.

The results suggest that microorganisms native to the *Myriophyllum* ecosystem, particularly those selected for their ability to attack specific plant tissues or, alternatively, amendments applied to the environment stimulating the growth of such populations, offer promising avenues for the biological control of these aquatic nuisance plants.



## Preface

This report presents preliminary results of a study of the cellulolytic or degenerating properties of enzymes produced by microorganisms occurring naturally on the surface tissues of two species of watermilfoil, *Myriophyllum spicatum* and *M. heterophyllum*.

The work was sponsored by the Civil Works Directorate of the Office, Chief of Engineers (DAEN-CW), through the U. S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP). Funds for the study were provided by DAEN-CW under Department of Army Appropriation No. 96X3122, Construction General. The APCRP is managed by the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The principal investigator for the work was Dr. Haim B. Gunner, Department of Environmental Sciences, University of Massachusetts, Amherst. He was assisted in the conduct of the study by Mr. Yuthana Limpa-Amara and Ms. Beryl Bouchard.

The study was monitored at WES by Dr. D. R. Sanders, Sr., Mr. R. F. Theriot, and Mr. E. A. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Laboratory (EL). The study was conducted under the general supervision of Dr. John Harrison, Chief, EL; Dr. C. J. Kirby, Chief, Environmental Resources Division, EL; and under the direct supervision of Dr. H. K. Smith, WTHG. Mr. J. L. Decell was Manager of the APCRP at WES.

Commanders and Directors of WES during the conduct of this study and preparation of the report were COL Nelson P. Conover, CE, and COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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## Contents

# MICROBIOLOGICAL CONTROL OF EURASIAN WATERMILFOIL

## Introduction

### Background

1. The severity of aquatic plant weed infestations has become a problem of growing national importance. Nuisance plants, such as *Myriophyllum spicatum*, the Eurasian watermilfoil which, a decade ago, had already succeeded in covering over 25,000 acres (61,776 ha) in eight Tennessee Valley Authority Reservoirs (Smith, Hall, and Stanley 1967), and *Hydrilla verticillata* and *Eichhornia crassipes* have, in recent years, burst explosively into the waterways of the southeastern United States (Allen 1975). Both *M. spicatum* and *M. heterophyllum*, a native milfoil, have emerged as significant aquatic nuisance plants in Massachusetts and nationwide, and have made the development of appropriate control procedures an urgent need. Herbicide formulations currently in use face increasing restrictions of their application so that a concomitant pressure is mounting for alternative control of pest species by the manipulation of biotic interactions.

2. Fungi have been reported to infest certain algae (Lund 1971) and bacteria have been isolated that kill or lyse a variety of green and blue-green algae (Stewart and Brown 1969, Daft and Stewart 1971, Shilo 1970, Granhall and Berg 1972). Controlling blue-green algal blooms by viruses probably offers a greater potential than other biological controls. Macrophytes have been reported subject to attack by a wide variety of pathogens including viruses, rusts, smuts, and other aquatic fungi (Zettler and Freeman 1972), but efforts to duplicate these natural attacks for management purposes are still in the investigatory stage or have been unsuccessful.

3. Charudattan (1975) has reported on, among others, intensive studies with *Rhizoctonia solani* Kuehn as a pathogen of waterhyacinth as well as work with *Pythium* sp. on hydrilla. Though effective in experimental plots, the larger question remains whether these pathogenic

fungi would function in an unrestricted environment and not eventually affect terrestrial crops. It must also be asked what effect artificial inoculation of pathogens into water would have on nonhost plants and animals, and, if microbial toxins are used, what effects their persistence would have on the environment.

Purpose and scope

4. In light of these questions, an alternative approach to biological control was proposed. Rather than introduce pathogenic microorganisms into the environment, we would isolate microorganisms native to the plant-dominated zone or phyllosphere of the aquatic plant and, by simple manipulation of their environment, induce them to produce enzymes lytic to selected tissues of the plant host. Subsequent to induction of these enzymes, the induced pathogens would be applied to the pest plant where pathogenesis would commence. The uniqueness of this approach lies in the exploitation of an intrinsic component of the ecosystem itself. The microorganism whose pathogenicity is employed for the control of the watermilfoil is, in fact, sponsored by the phyllosphere excretions of the plant host. Our approach, to temporarily induce pathogenesis, is based on no more than the environmental manipulation of a normally nonpathogenic microflora, at most saprophytic, whose pathogenic character ceases with the demise of its plant host. In this way, no new population is introduced into the ecosystem and no novel residues or environmental stresses accompany the control process.

Review of previous work

5. The presence of a phyllosphere effect, that is to say, the establishment of selected microfloral populations due to excretions from the host plant, is a well-established terrestrial phenomenon. In the terrestrial environment, where the focus has been on the root system or rhizosphere, the relationship is best described as protocooperation where both members benefit from the nonobligatory coexistence. Beneficial effects of the rhizosphere microflora on plant growth may be summarized as: (a) increased nutrient availablilty in the root zone (Estermann and McLaren 1961, Nicholas 1965, Rovira and Davey 1974);

(b) positive effects on nutrient absorption rates (Barber and Frankenburg 1971); (c) production of plant growth stimulators (Alexander 1977; Barea, Mavarro, and Montoya 1976; Katznelson and Bose 1959); and (d) increased resistance to soil-borne plant pathogens (Alexander 1977, Harris and Sommers 1968). Detrimental effects have also been shown to occur. These include the immobilization of limiting nutrients and the production of substances toxic to plant metabolism (Alexander 1977). More recently there has been growing interest in the rhizosphere of aquatic plants. Coler and Gunner (1969) found higher populations of bacteria and concentrations of amino acids surrounding the roots of free-floating duckweed. Mahmoud and Ibrahim (1970) found a positive rhizosphere effect with nitrifying bacteria, i.e., an increase in numbers, that increased with the age of submerged rice plants, and a negative rhizosphere effect, or decrease in numbers, with denitrifying bacteria. Many authors have reported the presence of nitrogen-fixing bacteria in the rhizosphere of aquatic plants. Patriquin and Knowles (1972) and Bristow (1974) found this is to be an area of higher populations and enhanced activity for nitrogen-fixing bacteria. The aquatic rhizosphere is thus becoming an important area of research with respect to the growth of submergent aquatic plants as well as food crops. Most recently, Blotnick, Rho, and Gunner (1980) have investigated the rhizosphere microflora of *M. heterophyllum* and found a significantly denser bacterial population in the extensively rooting systems of this plant, prompted, presumably, by greater access to organic nutrients in the sediments than in the surrounding lake water.

6. In the past, the manipulation of the rhizosphere has been used for plant protection rather than plant destruction. Koths and Gunner (1967) induced chitinase activity against *Fusarium roseum* in an *Arthrobacter* species isolated from the rhizosphere of carnations. Subsequent dissemination of this chitinolytic bacterium in the rhizosphere of the carnations protected against *Fusarium* attack. The rationale for this treatment was that the original isolate, endemic to the rhizosphere, would, in effect, be sponsored by the carnation and be helped to compete against other microorganisms in the environment. This

hypothesis was confirmed by the isolation of approximately 65 percent of the original inoculum numbers by the time of plant harvest. Thus, plant-dependent microorganisms would appear to offer an ecologically attractive reservoir from which to draw in developing biological control strategies.

## Materials and Methods

7. *Myriophyllum* spp. were originally collected from Pottapaug Pond, a spur of the Quabbin reservoir in western Massachusetts, and subsequently from Hampton Ponds in Westfield, Mass. Samples of *M. spicatum* were also provided from a site at Guntersville, Ala. Plants were sorted, washed in tap water, and maintained in aerated 10-gal (38-ℓ) aquaria illuminated with Sylvania Gro-lux standard 40-watt fluorescent plant lights, 2 lights per aquarium, for 16 hr each day.

8. In comparative survival trials under stress by various organisms, the plants were maintained in a modified Gerloff and Krombholtz solution (Andrews 1980). A variety of test regimes were conducted including the maintenance of five 10-cm-long shoots of *Myriophyllum* in 150-mℓ Erlenmeyer flasks containing 50 mℓ of the angiosperm growth medium and inoculated with various test organisms; the maintenance of individual 10-cm-long shoots anchored in an agar base in individual 15-mℓ test tubes filled with sterile medium; quart-size canning jars with five shoots per jar; and finally arrays of twenty to twenty-five 10- to 15-cm-long shoots anchored in washed, sterilized gravel in 38-ℓ aquaria.

9. Rhizoplane organisms, i.e. organisms bound to the plant surface, were obtained as described by Blotnick, Rho, and Gunner (1980) and maintained on nutrient agar (NA), trypticase soy agar (TSA), or trypticase soy broth (TSB; Difco). For sterilization experiments, plants were exposed for 30 sec to 5 min to 0.5, 10, and 25 percent hypochlorite, washed in sterile distilled water, and transferred to sterile culture solution. For exposure to antibiotics, myriophyllum tissue was suspended in 1 percent antibiotic solution for 6 hr followed by

12 successive washings in sterile distilled water. It was subsequently plated in Bacto-agar and observed for growth. Microwave sterilization was conducted with portions of plant placed in sterile vials and exposed for 3, 5, 8, 10, 13, 17, and 20 min. The plants were subsequently plated in Bacto-agar as a sterility check. Cellulose agar tubes were prepared in accordance with the method of Tansey (1971) and Stanton (unpublished data). To identify cellulolytic organisms, standard mineral salts medium was poured into the plates, allowed to harden, and an overlay of 1 percent carboxy-methyl cellulose and 0.02 percent urea applied. Cellulysis was evident in a clearing on the medium around active colonies (Figure 1). For inducing cellulase in growing cultures, No. Whatman Filter paper, ground in a Wiley Mill, was added to liquid mineral medium with a 0.02 percent urea supplement.

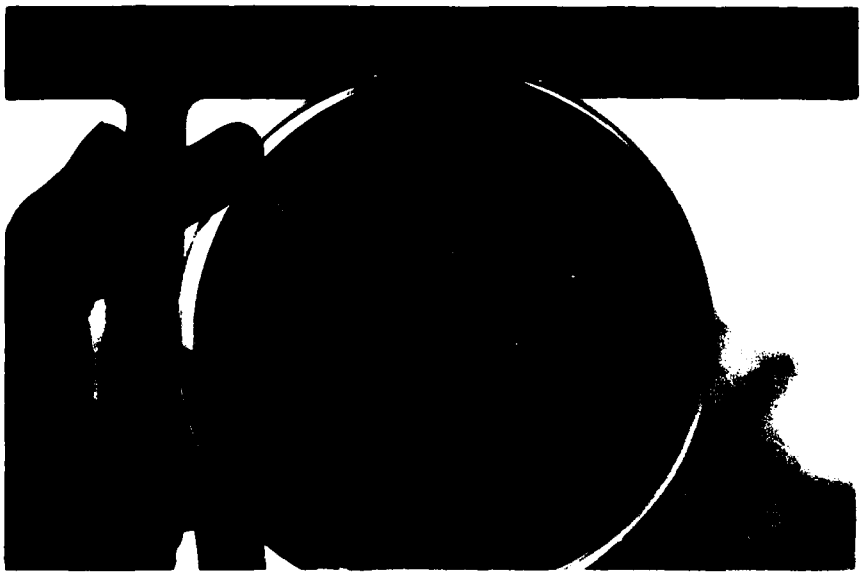

Figure 1. Isolation of cellulytic microorganisms by plating on a medium in which cellulose was the sole source of carbon. *Mycoleptodiscus terrestris* shown here leaves a clear zone as evidence of cellulose hydrolysis

10. Pectinolytic organisms were identified by growth in a medium prepared as follows:

| Combine in a Preheated Blender | per 500 mℓ |
|---|---|
| 1 N NaOH | 4.5 mℓ |
| 10% $CaCl_2 \cdot H_2O$ | 3.0 mℓ |
| Agar | 1.5 g |
| Yeast extract | 2.5 g |
| Casamino acids | 0.5 g |
| Boiling distilled $H_2O$ | 300 mℓ |

11. Add 10 g sodium polypectate and 200 mℓ boiling distilled $H_2O$ slowly and blend. Distribute in 250-mℓ flasks and autoclave.

12. Pectinolysis was determined by pitting of agar in petri plates (Figure 2). For enzyme enhancement, the same formulation was used and the semisolid medium reduced to liquid at maximum enzyme yield (Figure 3). Microbial inoculum was prepared so as to maximize enzyme

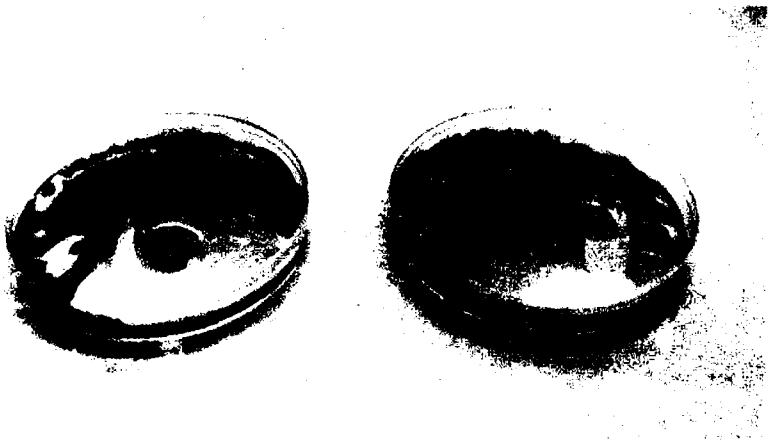

Figure 2. Isolation of pectinolytic microorganisms by plating on a medium on which pectin was the sole source of carbon. Pitting of the agar surface demonstrates pectinolytic activity

Figure 3. Liquification of solid pectin medium by a pectinolytic isolate after induction of enzymes

yield. *Mycoleptodiscus terrestris* was cultured for 14 days, culture Br-2 for 7 days, other bacterial isolates for 48 hr, and the Cyanobacteria for 22 days prior to inoculation. Inoculum was introduced at a rate from 0.14 to 1.4 mℓ per 100 mℓ of angiosperm medium, depending on the experiment.

13. For establishment of necrosis, a scoring system of 1-10 was established based on the discoloration and general decline of the plant as compared with untreated controls. Three weeks was generally considered the terminal point of experiments and plant death was achieved when no further development occurred from the growth points of the shoot section. Where possible, two independent observers tabulated results.

## Results

14. Screening of microorganisms associated with *Myriophyllum* spp. revealed that the principal flora present was bacterial with relatively

fewer fungi and actinomycetes present. *Cladosporium* and *Epicoccum* spp. appeared to grow on the plant surface. Gram negative rods, pseudomonad in character, grew in even tighter association with the plant. The latter proved extremely resistant to a variety of sterilization techniques including treatment with hypochlorite, amphyll, and streptomycin/penicillin washing, and even microwave sterilization. Indeed the plants proved virtually inseparable from their microbial resident, and under sustained sterilization ultimately died without relinquishing some fraction of this flora. Although no definitive attempt was made at taxonomic differentiation, morphological characterization and biochemical tests revealed a distribution of floral forms related to specific areas of the plant.

15. Of fundamental significance to this study was the initial demonstration that plant necrosis could be induced by stimulus to this naturally occurring flora on *Myriophyllum*, as shown by the data in Tables 1 and 2. In this instance, specimens of plants were incubated in sterile solutions of the defined mineral plant growth media. To these were added aliquots of sterilized pond water from the area from which the plants were harvested. Within 3 weeks, death of the test plants was observed with a complete browning and necrosis of the tissues. That this process is microbial and derives from the microflora uniquely associated with the plant was confirmed as follows: a transfer of solution was made from the original medium, presumably containing the pathobiological agents, microbial or chemical (PBA), to fresh test flasks containing *M. heterophyllum*, the sterile growth medium and sterilized pond water. This transfer resulted in necrosis and death of the plant within approximately 1 week. A transfer of the PBA medium from the second series to a series of flasks reduced the kill time to approximately 4 days (Table 1). It would appear that the pathogenic agents in these inoculi are members of the natural resident microflora on *M. heterophyllum* that have been induced to attack the plant tissue after the reduction of other resident microbial competitors, and possible stimulation by growth factors or nutrients in the sterile pond water. This contention was supported by further studies in which selected isolates from necrotic

*M. heterophyllum* tissue initiated the significantly earlier death of healthy *M. heterophyllum* (Table 2). These effects would appear to represent the generalized attack of the native phyllosphere microflora concentrated by passage through a medium in which competing aquatic forms had been eliminated. In effect, competition between potential pathogens and other native organisms reduces the ability of the potential pathogen to induce necrosis. By eliminating or reducing the competing populations, the pathogenic attack on the host may proceed with accordingly diminished constraints. That a mixture of isolates was more effective than certain of the isolates individually suggests that pathogenicity was enhanced by synergistic interactions, i.e., effects generated by interactions between two or more organisms which individuals cannot achieve alone, a process frequently observed in nature.

16. In confirmation of the foregoing, plants exposed to surface sterilization survived one third again as long as plants which were untreated (Table 3). This further demonstrated that the microflora intimately associated with the plants do, in fact, play a role in their decomposition, and isolates from the residual saprophytic flora were able to hasten decay rates (Table 4). It will be noted that the most significant and consistent results were provided by a cellulolytic fungus: cellulolysis subsequently appeared to provide a pathogenic mechanism to which the plants appeared particularly susceptible.

17. Indeed, some of the most telling results were achieved with the cellulolytic fungus subsequently identified as *Mycoleptodiscus terrestris* (Gerd.) Ostazeski when its cellulolytic ability was enhanced by successive passage through a medium containing cellulose as a sole source of carbon. As can be noted from Table 5, 75 percent mortality was achieved in 17 days and 100 percent mortality in 24 days.

18. An additional necrotic source was identified as a consortium of cyanobacteria and associated bacteria. In this case, the *Myriophyllum* species are enveloped by a veillike growth and reduced ultimately to a necrotic ball (Table 6). It is noteworthy that in both the application of cellulolytic fungus and the cyanobacterial cluster, the enhanced effects were due to the application of the induced pathogen. This would

suggest too that the flora normally inhabiting the *Myriophyllum* may exercise an enhancing saprophytic effect once a primary lesion has been effected by a pathogen. To characterize the necrotic process at the operative level, an attempt was made to establish the prevalence and pathogenic potential of cellulolytic and pectinolytic microorganisms among *Heterophyllum* spp.

19. Such isolates are not rare among the population inhabiting *Myriophyllum spicatum* as demonstrated in Table 7. Their pathogenic potential is shown by the decay of 70 to 100 percent of the *Myriophyllum spicatum* plants within 3 weeks. In addition, however, two other points of interest emerge: one is the relative specificity of the isolates to *Myriophyllum spicatum* (Figure 4)--these achieve only a 10 to 40 percent

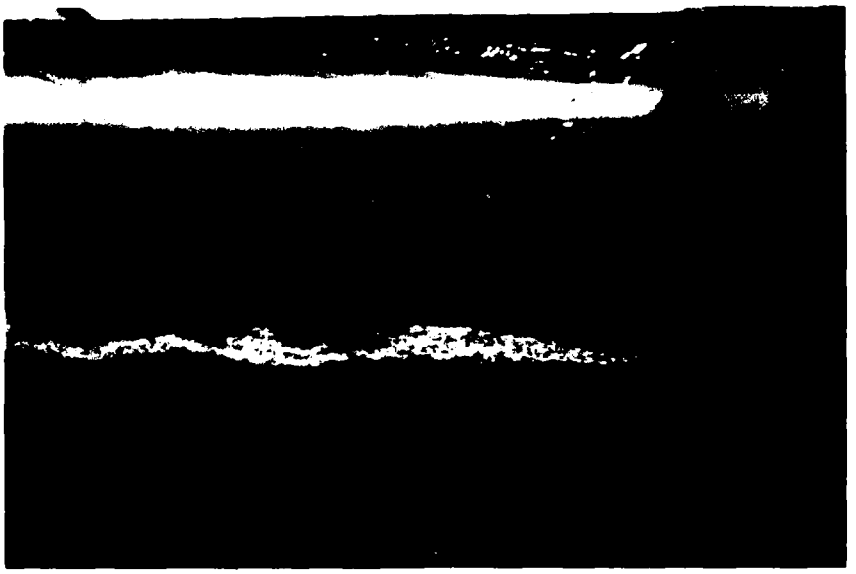

Figure 4. Selective attack of *Mycobacterium terrestris* on *M. spicatum* and survival of *M. heterophyllum* 28 days after inoculation

decay level when applied to *M. heterophyllum*. Second is the incitement to cellulolytic activity by the simple addition of increments of the sterile 1 percent cellulose medium to the test chambers. In this

instance, 80 percent of the *M. spicatum* plants decayed within 21 days and 40 percent of the *M. heterophyllum*. This would confirm the presence of a stable cellulolytic flora associated with these plants which might be induced to cellulolytic activity by the addition of appropriate amendments.

20. The acceleration of necrosis by the *Mycoleptodiscus terrestris* is shown in the data in Table 8. There is little suggestion of a dose-response relationship in the inoculum concentration employed, and the lowest inoculum (0.5 mℓ) achieved maximum decay. However, the addition of cellulose medium by itself stimulated significant necrosis, in this instance with a clear dose-response relationship between the level of cellulose additive and mortality, maximum decay being achieved with the highest concentration of inoculum. This would suggest that the greater availability of substrate enhances an accordingly increased growth of cellulolytic microflora.

21. The acceleration of decay of *Myriophyllum* spp. induced by the pectinolytic isolate Br-2 is shown in the data in Table 9. Again, the addition of sterile cellulose or pectin medium elicited a degree of acceleration in plant decay, greater with pectin than with cellulose and more so in the case of *M. spicatum* than with *M. heterophyllum* (Figures 5 and 6). The greatest effect was demonstrated by the application of the pectin or pectin-plus cellulose cultured organism, in which case 100 percent of the plant succumbed. Perhaps the most striking confirmation of the addition effect of growth media on the induction of populations inimical to watermilfoil may be seen in Table 10. In this instance, a cyanobacterial group which we had previously identified as an effective suppressant of milfoil, was compared with the effect achieved by the addition of aliquots of sterile angiosperm medium in which the cyanobacteria were cultured. As may be seen, both organisms and culture medium achieved 100 percent acceleration of necrosis of *M. spicatum*, while only the medium alone was as effective in the suppression of *M. heterophyllum*. Certainly, the mechanism of this interaction merits further investigation.

Figure 5. Selective attack of pectinolytic isolate Br-2 on *M. spicatum*

Figure 6. Uninoculated control *M. spicatum* and *M. heterophyllum*

## Discussion

22. The classic concept of biological control has been to seek out a parasite, predator, or pathogen that can be disseminated against the pest organism which it will unremittingly infect or devour. Such an approach which has its spectacular, if sometimes transient, successes must nonetheless be recognized as going against the ecological grain; i.e. the original absence of such a control agent from the natural ecosystem suggests that the natural balances are, or will eventually be, tipped against it. In this light, we have been prompted to test an alternative approach to biological control based on the manipulation of microbial communities naturally associated with *Myriophyllum* spp. and their immediate environment. Having early established that there is an extensive plant-associated microbial community, it soon became evident that, as might have been anticipated, it was members of this community who were the principal decomposers of the senescent plants. Representative bacteria, fungi, and even blue-green algae contributed to the necrotic attack. When stripped of this flora by various sterilizing techniques, the *Myriophyllum* extended its life by at least one third.

23. The question subsequently posed was, could this essentially saprophytic process be accelerated to true pathogenesis by selecting from the community of microbial decomposers isolates which, when cultured on the appropriate media, could be induced to generate enzymes lytic to selected plant tissues? The invariable presence of cellulose and pectin elements in plant tissue provided the rationale for the isolation and enhancement of enzyme yield of cellulolytic and pectinolytic isolates capable of bringing about the decline of exposed plants. The particular vulnerability of these target tissues was confirmed by the induction of an extensive cellulolytic and pectinolytic microflora simply by the respective addition of sterile cellulose and pectin media in test chambers which gave a significant increase in necrosis over untreated controls. Indeed, this is reminiscent of the classic practice of adding organic amendments to soil as a stimulus to rapidly growing

microorganisms readily able to utilize the substrates these amendments offer. The proliferation of these populations serves as a suppressant to fungal pathogens unable to compete with the faster growing organisms. The addition of pectin and cellulose would appear to selectively stimulate pectin- and cellulose-degrading organisms whose activities soon extend from the substrate provided to these materials in the plant itself. This would certainly suggest the merit of further examining the possible use of such amendments as a component of a control strategy.

24. There is a wide array of resident microorganisms on *Myriophyllum* which may be manipulated to speed the decay of these plants, yet it is noteworthy and in keeping with ecosystem specificity that there should be a much higher pathogenic potential of the isolates from *M. spicatum* to that species than to *M. heterophyllum*. It remains, of course, to be more broadly tested how narrowly specific induced pathogenesis remains; the results noted in the foregoing suggest that this may, in fact, reflect the tight bonding of specific microbial communities to their respective plant hosts and that this specificity may be of significance in potential plant control strategies.

25. Though a number of organisms have now been isolated from the flora associated with *Myriophyllum* spp., which have essentially been induced to temporarily make the leap from saprophytic to pathogenic behavior, the entire process of inducing death in the plant tissue must only, in part, be induced by the organism brought to temporary pathogenic behavior. Reinforcing its initiating role is the subsequent acceleration of necrosis by the decomposers naturally present on the plant. Though a number of active cellulose and pectin decomposers, and other nonspecific populations capable of inducing pathogenesis, have been isolated, it is clear that these represent only an initial screening and there remains the potential for even more effective control agents to be identified in the rich reservoir of decomposers.

26. These results point, as well, to the need to better understand the life cycle of the plant so that the optimum point in plant growth for infection to be initiated may be established. The necrotic process in the plant too requires close examination, e.g., the

physicochemical events of the plant inoculation strategies. Certainly these results already provide substantial grounds to perceive the plant ecosystem as the rational source of its own potential pathogens if only by way of a temporary extension of saprophytic behavior.

## Conclusions

27. No final conclusions can be drawn from this work thus far. However, the information obtained from this study is sufficiently promising to warrant the continuation of the search and evaluation of microorganisms for the biocontrol of the problem aquatic macrophyte, Eurasian watermilfoil.

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Table 1

Acceleration of Decomposition of *Myriophyllum heterophyllum* by Pathogens Associated with Necrotic Watermilfoil Tissue

| Treatment* | Viability,** days | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 8 | 22 | 28 |
| Control | + | + | + | ± | - |
| 1 | + | - | - | - | - |
| 2 | + | ± | - | - | - |

* Control - *Myriophyllum* in sterile pond water.
Treatment 1 - *Myriophyllum* in sterile pond water with 5 mℓ of PBA inoculum.
Treatment 2 - *Myriophyllum* surface sterilized with NaOCI in sterile pond water with 5 mℓ of PBA inoculum.

** + = alive and healthy; ± = deteriorating; - = dead.

Table 2

Accelerated Decomposition of *Myriophyllum heterophyllum* by Isolates from PBA Inoculum

| Treatment* | Viability,** days | | |
|---|---|---|---|
| | 0 | 9 | 18 |
| Control | + | + | ± |
| Isolate $K_5$ | + | ± | - |
| Isolate mixture $K_1$-$K_7$ | + | ± | - |

* Control - *Myriophyllum* in sterile pond water.
Isolate $K_5$ - *Myriophyllum* in sterile pond water with 5 mℓ inoculum of isolate $K_5$.
Isolate mixture $K_1$-$K_7$ - *Myriophyllum* in sterile water with 5 mℓ inoculum composed of equal parts of isolated $K_1$-$K_7$.
Isolates $K_1$, $K_2$, $K_3$, showed no effect by day 9 or 18.
Isolates $K_4$, $K_6$, $K_7$ showed some deterioration on day 18 but not before.

** + = alive and healthy; ± = deteriorating; - = dead.

Table 3

Decay of *Myriophyllum spicatum* and *Myriophyllum heterophyllum* in Angiosperm Medium

| Species | Average Decay Time, days* | |
|---|---|---|
| | Untreated Plants** | Surface-Sterilized Plants† |
| *Myriophyllum spicatum* | 40 | 60 |
| *Myriophyllum heterophyllum* | 25 | 40 |

* Approximate time at which 50 percent of plants were completely necrotic.
** Number of plants observed = 20.
† Number of plants observed = 10.

Table 4

Effect of Bacterial and Fungal Isolates and Cyanobacterial Consortium on Rate of Plant Decay*

| Inoculum | Rate of Decay** | |
|---|---|---|
| | *M. spicatum*† | *M. heterophyllum*† |
| Cellulolytic fungus BSF | ++ | ++ |
| Cyanobacterial consortium | + | + |
| Fungus YBF | + | ++ |
| Bacterium PB | 0 | ++ |
| Bacterium Sp | 0 | ++ |
| Bacterium PB | 0 | ++ |

* Bacteria, fungi, and cyanobacterial consortium isolated from decaying *M. spicatum* and *M. heterophyllum*.
** ++ = average decay time significantly less than uninoculated control plants.
+ = average decay time slightly less than uninoculated control plants.
0 = average decay time approximately same as or greater than uninoculated control plants.
† Plants surface sterilized.

Table 5

Effect of a Cellulolytic *Mycoleptodiscus* sp. (Strain BSF) on Decay of *Myriophyllum spicatum*

| | Percent Necrotic Plants, days | | | |
|---|---|---|---|---|
| Treatment | 0 | 10 | 17 | 24 |
| Control | 0 | 0 | 0 | 0 |
| BSF | 0 | 50 | 75 | 100 |

Table 6

Effect of a Cyanobacterial Consortium on Decay of *Myriophyllum spicatum*

| | Percent Necrotic Plants, days | | | |
|---|---|---|---|---|
| Treatment | 0 | 10 | 17 | 24 |
| Control | 0 | 0 | 0 | 25 |
| Cyanobacterial consortium | 0 | 0 | 100 | 100 |

Table 7

Acceleration of Decay of *Myriophyllum* spp. by Cellulolytic and Pectinolytic Microbial Isolates from *M. spicatum*

| | Percent Necrotic Plants, days* | | | | | |
|---|---|---|---|---|---|---|
| | *M. spicatum*** | | | *M. heterophyllum*** | | |
| Treatment | 7 | 14 | 21 | 7 | 14 | 21 |
| Control untreated | 0 | 0 | 0 | 0 | 0 | 0 |
| Control 1% cellulose medium | 0 | 70 | 80 | 10 | 40 | 40 |
| Br-3 | 10 | 40 | 70 | 20 | 20 | 20 |
| Br-4 | 20 | 30 | 70 | 0 | 0 | 0 |
| P-3 | 10 | 20 | 80 | 30 | 30 | 30 |
| P-4 | 0 | 40 | 90 | 40 | 30 | 30 |
| P-6 | 20 | 80 | 90 | 10 | 10 | 10 |
| P-7 | 60 | 80 | 100 | 0 | 10 | 10 |
| P-8 | 10 | 70 | 100 | 10 | 10 | 10 |
| Y-2 | 10 | 80 | 90 | 10 | 10 | 10 |
| Y-4 | 10 | 70 | 100 | 10 | 20 | 40 |
| Y-5 | 20 | 80 | 90 | 20 | 10 | 10 |

* Based on numerical scores: 1 = least decay, 10 = maximum decay.
** Number of plants observed, 10 per treatment.

Table 8

Acceleration of Necrosis of *Myriophyllum* spp. by *Mycoleptodiscus terrestris* and by Cellulose Induction Growth Medium

| Treatment | Inoculum size, mℓ | Percent Necrotic Plants, days* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *M. spicatum*** | | | *M. heterophyllum*** | | |
| | | 14 | 21 | 28 | 14 | 21 | 28 |
| Cellulose medium | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1.0 | 25 | 25 | 50 | 25 | 25 | 50 |
| | 2.0 | 25 | 50 | 75 | 25 | 25 | 50 |
| *M. terrestris* | 0.5 | 50 | 100 | 100 | 50 | 50 | 50 |
| | 1.0 | 50 | 75 | 75 | 25 | 25 | 25 |
| | 2.0 | 75 | 75 | 75 | 25 | 25 | 25 |

* Number of plants observed, 10 per treatment.
** Based on numberical scores: 1 = least decay, 10 = maximum decay.

Table 9

Acceleration of Decay of *Myriophyllum* spp. by Isolate BR-2, an Actinomycete Isolated from *M. spicatum*

| Treatment | Percent Necrotic Plants, days* | | | | | |
|---|---|---|---|---|---|---|
| | *M. spicatum*** | | | *M. heterophyllum*** | | |
| | 7 | 10 | 21 | 7 | 14 | 21 |
| Control untreated | 0 | 0 | 0 | 0 | 0 | 0 |
| Control 1% cellulose medium | 0 | 10 | 10 | 0 | 0 | 0 |
| Control 1% pectin medium | 10 | 40 | 50 | 20 | 30 | 40 |
| BR-2 grown on cellulose medium | 0 | 50 | 80 | 0 | 0 | 0 |
| Br-2 grown on pectin medium | 40 | 100 | 100 | 20 | 20 | 20 |
| Br-2 grown on cellulose, pectin medium combined | 70 | 100 | 100 | 10 | 10 | 20 |

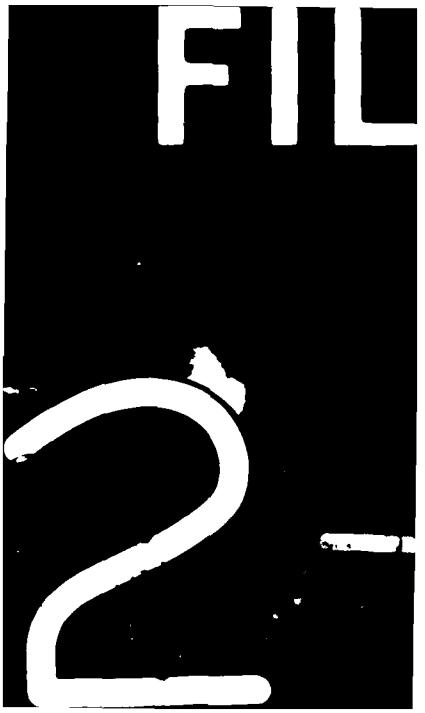
* Number of plants observed, 10 per treatment.
** Based on numerical scores: 1 = least decay, 10 = maximum decay.

Table 10

Acceleration of Necrosis of *Myriophllum* spp. by a Cyanobacterial Consortium and Angiosperm Growth Medium

| Treatment | Inoculum Size, mP | Percent Necrotic Plants, days* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *M. spicatum*** | | | *M. heterophyllum*** | | |
| | | 16 | 21 | 28 | 16 | 21 | 28 |
| Angiosperm medium | 0.5 | 25 | 50 | 75 | 25 | 50 | 75 |
| | 1.0 | 75 | 75 | 75 | 25 | 25 | 50 |
| | 2.0 | 50 | 100 | 100 | 0 | 75 | 100 |
| Cyanobacterial consortium | 0.5 | 25 | 25 | 25 | 25 | 75 | 75 |
| | 1.0 | 25 | 50 | 25 | 0 | 25 | 25 |
| | 2.0 | 100 | 100 | 100 | 25 | 50 | 50 |

* Number of plants observed, 10 per treatment.
** Based on numerical scores: 1 = least decay, 10 = maximum decay.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